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Gray-Matter Volume, Midbrain Dopamine D2/D3 Receptors and Drug Craving in Methamphetamine Users

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Abstract

Dysfunction of the mesocorticolimbic system plays a critical role in clinical features of addiction. Despite evidence suggesting that midbrain dopamine receptors influence amphetamine-induced dopamine release and that dopamine is involved in methamphetamine-induced neurotoxicity, associations between dopamine receptors and gray-matter volume have been unexplored in methamphetamine users. Here we used magnetic resonance imaging and [¹⁸F]fallypride positron emission tomography, respectively, to measure gray-matter volume (in 58 methamphetamine users) and dopamine D2/D3 receptor availability (binding potential relative to nondisplaceable uptake of the radiotracer, BP_{ND}) (in 31 methamphetamine users and 37 control participants). Relationships between these measures and self-reported drug craving were examined. Although no difference in midbrain D2/D3 BP_{ND} was detected between methamphetamine and control groups, midbrain D2/D3 BP_{ND} was positively correlated with gray-matter volume in the striatum, prefrontal cortex, insula, hippocampus and temporal cortex in methamphetamine users, but not in control participants (group-by-midbrain D2/D3 BP_{ND} interaction, $p < 0.05$ corrected for multiple comparisons). Craving for methamphetamine was negatively associated with gray-matter volume in the insula, prefrontal cortex, amygdala, temporal cortex, occipital cortex, cerebellum, and thalamus ($p < 0.05$ corrected for multiple comparisons). A relationship between midbrain D2/D3 BP_{ND} and methamphetamine craving was not detected. Lower midbrain D2/D3 BP_{ND} may increase vulnerability to deficits in gray-matter volume in mesocorticolimbic circuitry in methamphetamine

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Conflict of Interest

The authors declare no conflict of interest.

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users, possibly reflecting greater dopamine-induced toxicity. Identifying factors that influence prefrontal and limbic volume, such as midbrain BP_{ND}, may be important for understanding the basis of drug craving, a key factor in the maintenance of substance use disorders.

Keywords

D2/D3 dopamine receptors; craving; gray-matter volume; methamphetamine; ventral tegmental area; substantia nigra

Introduction

A variety of studies have indicated that exposure to methamphetamine is associated with abnormalities in the mesocorticolimbic pathway. These include deficits in tyrosine hydroxylase (1, 2), dopamine transporter molecules (1, 3), and dopamine D2/D3 receptor availability (4–6). Furthermore, methamphetamine users who relapse during treatment have lower levels of dopamine D2/D3 receptor availability and dopamine release in the striatum than those who maintain abstinence (7), suggesting that dopamine dysfunction may be clinically relevant. Chronic methamphetamine exposure is also associated with structural abnormalities in dopamine terminal fields, with lower gray-matter volume in the caudate nucleus, anterior cingulate, prefrontal cortex, insula, amygdala and hippocampus in methamphetamine users than healthy controls (8–11). Despite evidence that methamphetamine-induced changes in dopaminergic markers are correlated with changes in gray-matter volume (12), these associations have not been tested in humans. Understanding the mechanisms that underlie both neurochemical and structural deficits in mesocorticolimbic circuitry has the potential to help clarify addiction-related symptoms, such as drug craving, which persist in abstinent stimulant users (13).

Methamphetamine-induced damage of dopaminergic neurons and neighboring cells has been linked to excessive levels of intracellular and intrasynaptic dopamine (14–16). Methamphetamine increases cytosolic levels of dopamine, which is degraded to form oxidative metabolites that damage dopamine nerve terminals (17). Mice lacking dopamine transporters (DAT knockouts) exhibit degeneration of GABAergic neurons in the striatum (18), providing evidence that excessive extracellular dopamine can be neurotoxic. Furthermore, administration of D1- and D2-type receptor antagonists attenuates methamphetamine-induced loss of dopamine transporters and tyrosine hydroxylase as well as apoptosis in the striatum (19). In humans, lower dopamine D2/D3 receptor availability in the midbrain, which has a high proportion of somatodendritic dopamine autoreceptors (20), has been associated with greater amphetamine-induced striatum dopamine release (21). This work suggests that regulation of dopamine release by somatodendritic dopamine autoreceptors in the substantia nigra and ventral tegmental area (22) may influence methamphetamine-induced neurotoxicity.

Dopamine signaling and brain function in regions that receive dopaminergic projections from midbrain have also been linked to drug craving. For example, cue-induced cocaine craving has been correlated with striatal dopamine release (23, 24), and drug-related cues elicit changes in brain activation in the orbitofrontal cortex, anterior cingulate (25–29),

ventral striatum (25), amygdala (26, 27, 30, 31), and insula (25). Self-reported craving in these studies is positively correlated with activation in the striatum, orbitofrontal cortex, dorsolateral prefrontal cortex, and anterior cingulate cortex (32). A negative correlation between volume of the right inferior frontal gyrus and methamphetamine craving has been noted (33); but associations of methamphetamine craving with gray-matter volume in other brain regions that receive dopaminergic innervation and/or with midbrain dopamine receptors have not been tested.

The goal of the current study was to examine the relationships between dopamine D2/D3 receptor availability (binding potential relative to nondisplaceable uptake of the radiotracer; BP_{ND}) using positron emission tomography (PET), gray-matter volume measured using high-resolution structural magnetic resonance imaging (MRI), and self-reported craving for methamphetamine. Previous studies have shown lower striatal dopamine D2/D3 BP_{ND} in methamphetamine users than in healthy controls (4–6); a similar difference was expected regarding the midbrain. As indicated above, evidence suggests that excessive intrasynaptic dopamine is neurotoxic (14–16) and that midbrain D2/D3 BP_{ND} is negatively correlated with amphetamine-induced striatal dopamine release (21). It was therefore anticipated that in methamphetamine users, but not in controls, lower midbrain D2/D3 BP_{ND} would be associated with smaller gray-matter volume in brain regions that receive dopaminergic projections from the midbrain. Since methamphetamine-induced damage may similarly influence both dopamine receptors and morphometry in brain regions that receive dopaminergic innervation, a positive association between striatal D2/D3 BP_{ND} and striatal gray-matter volume in methamphetamine users was predicted. Furthermore, it was expected that lower midbrain D2/D3 BP_{ND} and lower gray-matter volumes in regions of the mesocorticolimbic system would be associated with greater methamphetamine craving.

Methods and Materials

Participants

Ninety-five participants (58 methamphetamine users, 37 healthy controls) were included in this study. Participants were recruited through online and print advertisements, received a detailed explanation of the study (as approved by the UCLA Institutional Review Board), and gave written informed consent. Data from a subset of these participants were included in previous publications comparing methamphetamine and control participants on gray-matter volume (8) and striatal D2/D3 BP_{ND} (4); this study does not focus on previously reported group differences. A previous publication also used a subset of these participants to examine the correlation between methamphetamine craving and gray-matter volume in the right inferior frontal gyrus (33). Here, we extend this analysis to the whole-brain assessments of gray-matter volume and midbrain D2/D3 BP_{ND}.

Exclusion criteria were as follows: central nervous system, cardiovascular, pulmonary, or systemic disease; use of psychotropic medications, loss of consciousness >30 min, HIV seropositive status, and pregnancy. The Structured Clinical Interview for DSM-IV (34) was used to determine current Axis I disorders. Control participants had no current axis I disorders except nicotine dependence. In addition to nicotine dependence, some methamphetamine users met criteria for current marijuana dependence (n=4) or panic

disorder (n=1). Methamphetamine users participated on a residential basis at the UCLA General Clinical Research Center (GCRC) and tested positive for methamphetamine in urinalysis at study entry. After admission, abstinence from methamphetamine, cocaine, opiates, marijuana, and benzodiazepines was verified by urine testing. MRI and PET scanning were conducted during the first 4–7 days of abstinence from methamphetamine. Control subjects participated on an outpatient basis, and tested negative for methamphetamine, cocaine, opiates, marijuana, and benzodiazepines on scan days.

Self-Report of Drug Use and Craving

Drug use and demographic variables were collected using a drug-use survey prepared for this study. Methamphetamine craving for was assessed at intake by self-report. Specifically, participants were instructed to select the multiple of 10 between 0 (“not at all”) and 100 (“strongest ever”), that best corresponded with their craving for methamphetamine in “the past 24 hours.” At intake, all methamphetamine users reported using methamphetamine within the last 5 days.

MRI Acquisition

A magnetization-prepared rapid acquisition gradient echo sequence (TR=1900 ms, TE=4.38 ms, flip angle=15°, FOV=256×256×160, 160 slices, thickness: 1-mm) was run on a 1.5-Tesla Siemens Sonata scanner (Erlangen, Germany) with a standard quadrature head coil to produce high-resolution, T1-weighted, MRIs.

MRI Preprocessing

As outlined previously (8), images were aligned to a standardized stereotactic space with the sagittal plane serving as the yz-plane, the axial-oblique plane normal to this and containing the anterior and posterior commissures (AC-PC plane) as the xy-plane, and the coronal-oblique plane normal to the sagittal AC-PC planes serving as the xz-plane. The origin of the space was set at the left-right and inferior-superior midpoint of the anterior commissure.

The voxel-based morphometry (35) toolbox (<http://dbm.neuro.uni-jena.de/vbm/>) for SPM8 (Wellcome Department of Imaging Neuroscience, London) running on MATLAB® 7.9 (Mathworks, Sherborn, MA, USA) was used to preprocess images. As described previously (8), AC-PC aligned images are de-noised using an optimized block-wise non-local means de-noising filter (36). Images were segmented into three classifications (gray matter, white matter, and cerebrospinal fluid) using an adaptive maximum *a posteriori* technique (37) including partial-volume estimation (38). A hidden Markov Random Field approach (39) was used to denoise the data. Each image was registered to a standard template in Montreal Neurological Institute (MNI) space using Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (40).

The images were modulated by a procedure in which the intensity value of each voxel was multiplied by the local value of the Jacobian determinant of the deformation used to register each brain to MNI space. Linear components of the deformations, reflecting scaling due to head size, were not considered during modulation so that differences in volume due to head size would not affect intensity values. Intensity at each voxel (“gray-matter volume”)

reflected both the probability that the voxel contained gray matter and local deformations used to register the brain to MNI space. Finally images were smoothed using an 8-mm full-width half maximum (FWHM) Gaussian kernel.

PET Acquisition

Dopamine D2/D3 receptor availability was assayed using [^{18}F]fallypride (41) and a Siemens ECAT EXACT HR+ scanner [in-plane resolution FWHM=4.6 mm, axial FWHM=3.5 mm, axial field of view=15.52 cm] in three-dimensional mode. A 7-min transmission scan was acquired using a rotating $^{68}\text{Ge}/^{68}\text{Ga}$ rod source for attenuation correction. PET dynamic data acquisition was initiated with a bolus injection of [^{18}F]fallypride (~5 mCi in 30 s). Emission data were acquired in two 80-min sessions, separated by a break to minimize participant discomfort and allow the participant to urinate to reduce radiation exposure to the bladder wall. Data were reconstructed using ECAT v7.3 OSEM (Ordered Subset Expectation Maximization; 3 iterations, 16 subsets) after corrections for decay, attenuation, and scatter.

PET Preprocessing

Reconstructed PET data were combined into 16 sequential images, each containing an average of 10-min of data. PET images were corrected for head motion using rigid-body transformation with FSL FLIRT. Co-registration of the second PET image to the structural MRI using the ART software package was computed using a six-parameter rigid-body transformation and subsequently applied to all 16 PET images in the series (42).

A midbrain volume of interest (VOI), encompassing the ventral tegmental area and substantia nigra, was delineated using previously described methods (43). Bilateral caudate, putamen and nucleus accumbens VOIs were created using FSL FIRST (<http://www.fmrib.ox.ac.uk/fsl/first/index.html>) (44). Time-radioactivity data from VOIs were extracted from the motion-corrected, co-registered images and imported into PMOD 3.2 for kinetic modeling (PMOD Technologies Ltd., Zurich, Switzerland). Time-radioactivity curves were fit using the simplified reference-tissue model (SRTM; 45); to provide an estimate of k_2' , the rate parameter for transfer of the tracer from the reference region to the plasma. As the cerebellum is nearly devoid of measurable specific binding sites for the radiotracer, a cerebellar VOI was used as a reference region (46). A volume-weighted average of k_2' estimates from high-radioactivity regions (i.e., the caudate and putamen) was computed. The time-radioactivity curves were refit using the SRTM2 model (47) with the computed k_2' value held constant across all VOIs. BP_{ND} was then calculated by subtracting 1.0 from the product of the tracer delivery (R_1) and the tracer washout (k_2'/k_2a).

Statistical Analyses

Demographic and drug-use data were analyzed in SPSS 21 (Armonk, NY: IBM Corp). As appropriate, Student's t-tests and Chi-square tests were used to evaluate group differences in these variables. Bivariate correlations and Student's t-tests were used to examine the relationships between methamphetamine craving and the demographic variables (Table 1). Univariate analysis of covariance (ANCOVA), with midbrain D2/D3 BP_{ND} as the dependent variable, group as a between-subjects factor, and age, sex, smoking status, and frequency of marijuana use as covariates, was used to examine group differences in midbrain D2/D3

BP_{ND}. Partial correlations between methamphetamine craving and midbrain or striatal D2/D3 BP_{ND} included sex, days since last methamphetamine use, and frequency of methamphetamine use as covariates.

To test the group-by-midbrain D2/D3 BP_{ND} interaction on gray-matter volume, FSL's Randomise (<http://www.fmrib.ox.ac.uk/fsl/randomise>, version 5.1) was used to implement voxelwise permutation-based nonparametric inference for ANCOVA with age, sex, smoking status and frequency of marijuana use as covariates. A second model with the same covariates was used to test the group-by-striatal D2/D3 BP_{ND} interaction on gray-matter volume. The relationship between methamphetamine craving and gray-matter volume was examined using nonparametric inference for linear regression including sex, days since last methamphetamine use, and frequency of methamphetamine use as covariates. For each of the contrasts tested, 10,000 permutations were performed. Threshold-free cluster enhancement was used to correct for multiple comparisons, and a statistical threshold of $p < 0.05$ was applied to each of the resulting statistical maps.

Results

Participant Characteristics

The subset of participants in the methamphetamine ($n=31$) and control groups ($n=37$) with assessments of dopamine D2/D3 BP_{ND} did not differ in age ($t(66)=1.69$, $p=0.10$), sex (Chi-Square (68)=1.87, $p=0.17$), days of alcohol use in the 30 days preceding study entry ($t(66)=0.088$, $p=0.93$), number of alcoholic drinks per week ($t(66)=0.41$, $p=0.68$), or pack-year smoking history ($t(66)=-1.33$, $p=0.19$). The methamphetamine group smoked marijuana more frequently ($t(66)=-3.0$, $p=0.004$) and included more tobacco cigarette smokers than the control group (Chi-Square (68)=5.81, $p=0.02$). On average, participants in the methamphetamine group ($n=58$) used methamphetamine on 22.1 ± 7.8 days in the 30 days preceding study entry and had used methamphetamine heavily for 7.9 ± 6.8 years (heavy use defined as 3 times a week or at least one 2-day binge per week) (Table 1).

Relationships between Gray-Matter Volume and Midbrain or Striatal D2/D3 BP_{ND}

Methamphetamine and control groups did not differ in midbrain D2/D3 BP_{ND} ($F(1,62)=0.76$, $p=0.39$, Figure 1; control group mean: 1.11, standard deviation: 0.34; methamphetamine group mean: 1.13, standard deviation: 0.26). Whole-brain, voxelwise analysis revealed significant group-by-midbrain D2/D3 BP_{ND} interactions on gray-matter volume of bilateral striatum, insula, temporal cortex, left dorsolateral prefrontal cortex, hippocampus, orbitofrontal cortex, and right inferior frontal cortex (Figure 2A, Supplemental Table 1). In the methamphetamine group, midbrain D2/D3 BP_{ND} was positively associated with gray-matter volume in bilateral striatum, amygdala, hippocampus, temporal pole, inferior frontal gyrus, dorsolateral prefrontal cortex, and insula (Figure 2B, Supplemental Table 1), while no significant associations between gray-matter volume and midbrain D2/D3 BP_{ND} were detected in the control group (p 's > 0.4 ; see Supplemental Figures 2 and 3 for scatter plots and effect size maps).

Midbrain and striatal D2/D3 BP_{ND} were positively correlated in both methamphetamine ($r(31)=0.63$, $p<0.001$) and control groups ($r(37)=0.72$, $p<0.001$). Therefore, we examined the correlation between striatal D2/D3 BP_{ND} and gray-matter volume across the whole brain. Group-by-striatal D2/D3 BP_{ND} interactions were detected on gray-matter volume of bilateral striatum, left pallidum, and temporal cortex (Supplemental Table 2, Supplemental Figure 4), reflecting positive correlations between striatal D2/D3 BP_{ND} and gray-matter volume in the methamphetamine group, but no significant correlations in the control group. As both striatal and midbrain D2/D3 BP_{ND} were associated with bilateral striatal volume in the methamphetamine group, we examined whether D2/D3 BP_{ND} in each region was uniquely associated with striatal volume in a shared model. In the methamphetamine group, linear regression showed that striatal ($\beta=0.38$, $t(24)=3.00$, $p=0.007$) and midbrain D2/D3 BP_{ND} ($\beta=0.28$, $t(24)=2.30$, $p=0.03$) were both significant predictors of striatal gray-matter volume when included in the same statistical model along with age, gender, smoking status, and frequency of marijuana use as covariates (adjusted $R^2=0.759$, $F(6,24)=16.75$, $p<0.001$; Figure 3). In the control group, striatal gray-matter volume was not associated with striatal ($\beta=-0.18$, $t(30)=-0.68$, $p=0.50$) or midbrain D2/D3 BP_{ND} ($\beta=-0.02$, $t(30)=-0.07$, $p=0.95$) in a linear regression that also included age, sex, smoking status, and frequency of marijuana use as covariates (adjusted $R^2=0.150$, $F(6,30)=2.01$, $p=0.09$).

Relationships of Methamphetamine Craving to Dopamine D2/D3 BP_{ND} and Gray-Matter Volume

Self-reported craving was not correlated with age ($r(58)=-0.11$, $p=0.43$), frequency of alcohol use ($r(58)=-0.005$, $p=0.97$), number of alcoholic drinks per week ($r(58)=-0.10$, $p=0.46$), frequency of marijuana use ($r(58)=-0.04$, $p=0.78$), lifetime cigarette exposure (pack-years) ($r(58)=-0.13$, $p=0.32$), or years of heavy methamphetamine use ($r(55)=-0.02$, $p=0.88$). The index, days since last methamphetamine use, was positively associated with craving ($r(58)=-0.36$, $p=0.006$), and women reported stronger craving than men ($t(56)=-2.19$, $p=0.03$). Furthermore, there was a trend for those who used methamphetamine more often (days of use in the last 30 days) to report stronger craving ($r(58)=0.25$, $p=0.06$); therefore, these variables were included as covariates in statistical analyses examining the relationship of methamphetamine craving to gray-matter volume or dopamine D2/D3 BP_{ND}. No statistically significant relationships between midbrain ($r(24)=0.01$, $p=0.95$) or striatal D2/D3 BP_{ND} ($r(24)=-0.12$, $p=0.55$) and methamphetamine craving were detected ($n=29$). In contrast, whole-brain voxelwise analysis revealed that methamphetamine craving was negatively correlated with gray-matter volume in bilateral insula, orbitofrontal cortex, rostral anterior cingulate, lateral prefrontal cortex, lateral occipital cortex, parietal cortex, cerebellum and thalamus; and in left paracingulate gyrus ($p<0.05$ corrected; Figure 4, Supplemental Table 3, see Supplemental Figures 5 and 6 for scatter plots and effect size maps).

Discussion

This work extends previous findings of structural brain abnormalities associated with chronic methamphetamine use by demonstrating that in methamphetamine users, gray-matter volume varies with midbrain and striatal D2/D3 BP_{ND} and with methamphetamine

craving. Since the midbrain has a high proportion of dopamine autoreceptors, the positive relationship between midbrain D2/D3 BP_{ND} and gray-matter volume in the methamphetamine group but not in the control group, suggests that capacity for regulating dopamine signaling may influence methamphetamine-induced neurotoxicity in brain regions that receive dopaminergic projections from the midbrain. Methamphetamine-induced toxicity may have a similar influence on both dopaminergic markers and brain morphology in the striatum (12), consistent with the positive association between striatal D2/D3 BP_{ND} and striatal gray-matter volume detected in the methamphetamine group, but not in the control group. These associations remained significant in the methamphetamine group after including midbrain D2/D3 BP_{ND} as a covariate, suggesting that additional factors that influence the extent of neurotoxicity or that predate drug use influence the association between striatal BP_{ND} and gray-matter volume in methamphetamine users.

An emerging literature suggests that the regulation of dopamine release, synthesis, and firing rate by dopamine D2/D3 autoreceptors (22) plays an important role in addiction. For example, mice lacking D2 autoreceptors are more sensitive to the rewarding effects of cocaine than wild-type mice, as indexed by a conditioned place preference procedure (48). Studies of healthy individuals show that impulsivity and novelty seeking, personality traits associated with vulnerability for addiction (49–52), are negatively correlated with midbrain D2/D3 BP_{ND} (21, 53).

More work is necessary to determine how methamphetamine exposure influences dopamine receptors in the midbrain. A study in rodents demonstrated that amphetamine self-administration attenuates D2 autoreceptor function (54). Since D2 autoreceptors inhibit the firing of dopamine neurons and dopamine synthesis and release, this finding may help explain why resting-state functional connectivity of the midbrain to the mesocorticolimbic system is greater in methamphetamine users than control participants (55). Although methamphetamine and control groups did not differ in midbrain D2/D3 BP_{ND}, it is possible that this finding reflects some nonspecificity of the radiotracer used. [¹⁸F]fallypride binds to both D2 and D3 receptors with nearly equal affinity (56), and a study using the D3 receptor-preferring ligand [¹¹C]-(+)-propylhexahydro-naphtho-oxazin showed that D3 receptor availability was higher in the substantia nigra of methamphetamine users than controls (57). D2 and D3 receptors are both found in midbrain (58), therefore, it is possible that D2 receptor deficits in the midbrain are masked by elevated D3 binding (57).

Although midbrain D2/D3 BP_{ND} was correlated with gray-matter volume in brain regions that are also associated with methamphetamine craving, no direct association between craving and midbrain D2/D3 BP_{ND} was detected. While dopamine release triggered by conditioned cues is correlated with craving (23, 24), methylphenidate-induced increases in striatal dopamine do not produce craving unless methylphenidate is paired with drug cues (59), suggesting that dopamine signaling in the striatum is not sufficient to produce drug craving. Based on studies in animals showing that engagement of frontal-mesencephalic, amygdala-mesencephalic, and frontal-striatal pathways is necessary for the reinstatement or drug-seeking behavior (60, 61), it has been hypothesized that engagement of these circuits is also important for eliciting drug craving in humans (59). These findings suggest that

assessments of neurochemical signaling from frontal and limbic regions to midbrain and/or striatum may be more useful for predicting individual differences in drug craving.

Previous studies have revealed differences between healthy and methamphetamine-dependent individuals in gray-matter morphometry in striatum, insula, and prefrontal cortex (8–10, 62); this study extends these results by linking these deficits to a feature of methamphetamine use disorder. Methamphetamine craving was negatively correlated with gray-matter volume in a distributed set of brain regions that have consistently been implicated in functional (32) and structural brain studies of craving for other drugs (63, 64). Functional MRI and PET studies show that greater self-reported cue-induced craving is positively correlated with activation in the striatum, and orbitofrontal, dorsolateral prefrontal and anterior cingulate cortices (32). There are many functional and structural connections between these regions (65) and signaling through these pathways may remain intact or perhaps even heightened in response to drug-related stimuli. In contrast, methamphetamine users display less activation in prefrontal and anterior cingulate cortices than healthy controls while engaging in tasks that require self-control (66, 67). As less self-control is associated with greater methamphetamine craving (33), future studies might combine structural and functional MRI to determine if deficits in gray-matter volume disrupt functional connectivity in circuitry relevant for self-control and drug craving.

Methodological limitations associated with self-report measurements of craving warrant mentioning. In this study, participants retrospectively rated their craving for methamphetamine in the 24 hours before testing; but little is known about the temporal dynamics of craving (68). Individuals included in this study were made aware that participation required maintaining abstinence from methamphetamine for at least 7 days; and the circumstances surrounding study participation may have influenced craving levels. It is possible that more naturalistic assessments of methamphetamine craving may produce different results.

This study provides new evidence that midbrain D2/D3 BP_{ND} is related to gray-matter volume in methamphetamine users, suggesting that dopamine autoreceptors may play a role in limiting methamphetamine-induced toxicity; however, many interrelated mechanisms, such as oxidative stress, excitotoxicity, inflammation, mitochondrial function, hyperthermia, and disruption of the blood brain barrier contribute to methamphetamine-induced cell damage (17, 69). More work is necessary to determine how these neurotoxic mechanisms, genetic variability and other environmental factors influence the MRI signal through changes in synaptogenesis, dendritic arborization, or cell numbers. Identifying the mechanisms that produce abnormalities in gray-matter volume in methamphetamine users may prove useful for developing interventions that lessen symptoms of drug use disorders, such as craving.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Wilson JM, Kalasinsky KS, Levey AI, Bergeron C, Reiber G, Anthony RM, et al. Striatal dopamine nerve terminal markers in human, chronic methamphetamine users. *Nat Med.* 1996 Jun; 2(6):699–703. [PubMed: 8640565]
2. Hotchkiss AJ, Morgan ME, Gibb JW. The long-term effects of multiple doses of methamphetamine on neostriatal tryptophan hydroxylase, tyrosine hydroxylase, choline acetyltransferase and glutamate decarboxylase activities. *Life Sci.* 1979 Oct 15; 25(16):1373–1378. [PubMed: 42834]
3. Volkow ND, Chang L, Wang GJ, Fowler JS, Leonido-Yee M, Franceschi D, et al. Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. *Am J Psychiatry.* 2001 Mar; 158(3):377–382. [PubMed: 11229977]
4. Lee B, London ED, Poldrack RA, Farahi J, Nacca A, Monterosso JR, et al. Striatal dopamine d2/d3 receptor availability is reduced in methamphetamine dependence and is linked to impulsivity. *J Neurosci.* 2009 Nov 25; 29(47):14734–14740. [PubMed: 19940168]
5. Groman SM, Lee B, Seu E, James AS, Feiler K, Mandelkern MA, et al. Dysregulation of D(2)-mediated dopamine transmission in monkeys after chronic escalating methamphetamine exposure. *J Neurosci.* 2012 Apr 25; 32(17):5843–5852. [PubMed: 22539846]
6. Volkow ND, Chang L, Wang GJ, Fowler JS, Ding YS, Sedler M, et al. Low level of brain dopamine D2 receptors in methamphetamine abusers: association with metabolism in the orbitofrontal cortex. *Am J Psychiatry.* 2001 Dec; 158(12):2015–2021. [PubMed: 11729018]
7. Wang GJ, Smith L, Volkow ND, Telang F, Logan J, Tomasi D, et al. Decreased dopamine activity predicts relapse in methamphetamine abusers. *Mol Psychiatry.* 2012 Sep; 17(9):918–925. [PubMed: 21747399]
8. Morales AM, Lee B, Hellemann G, O'Neill J, London ED. Gray-matter volume in methamphetamine dependence: cigarette smoking and changes with abstinence from methamphetamine. *Drug Alcohol Depend.* 2012 Oct 1; 125(3):230–238. [PubMed: 22445480]
9. Schwartz DL, Mitchell AD, Lahna DL, Luber HS, Huckans MS, Mitchell SH, et al. Global and local morphometric differences in recently abstinent methamphetamine-dependent individuals. *Neuroimage.* 2010 May 1; 50(4):1392–1401. [PubMed: 20096794]
10. Thompson PM, Hayashi KM, Simon SL, Geaga JA, Hong MS, Sui Y, et al. Structural abnormalities in the brains of human subjects who use methamphetamine. *J Neurosci.* 2004 Jun 30; 24(26):6028–6036. [PubMed: 15229250]
11. Orikabe L, Yamasue H, Inoue H, Takayanagi Y, Mozue Y, Sudo Y, et al. Reduced amygdala and hippocampal volumes in patients with methamphetamine psychosis. *Schizophr Res.* 2011 Nov; 132(2–3):183–189. [PubMed: 21784619]
12. Groman SM, Morales AM, Lee B, London ED, Jentsch JD. Methamphetamine-induced increases in putamen gray matter associate with inhibitory control. *Psychopharmacology (Berl).* 2013 Oct; 229(3):527–538. [PubMed: 23748383]
13. Zorick T, Nestor L, Miotto K, Sugar C, Hellemann G, Scanlon G, et al. Withdrawal symptoms in abstinent methamphetamine-dependent subjects. *Addiction.* 2010 Oct; 105(10):1809–1818. [PubMed: 20840201]
14. Ares-Santos S, Granado N, Moratalla R. The role of dopamine receptors in the neurotoxicity of methamphetamine. *J Intern Med.* 2013 May; 273(5):437–453. [PubMed: 23600399]
15. Bozzi Y, Borrelli E. Dopamine in neurotoxicity and neuroprotection: what do D2 receptors have to do with it? *Trends Neurosci.* 2006 Mar; 29(3):167–174. [PubMed: 16443286]

16. Riddle EL, Fleckenstein AE, Hanson GR. Mechanisms of methamphetamine-induced dopaminergic neurotoxicity. *AAPS J.* 2006; 8(2):E413–E418. [PubMed: 16808044]
17. Yamamoto BK, Bankson MG. Amphetamine neurotoxicity: cause and consequence of oxidative stress. *Crit Rev Neurobiol.* 2005; 17(2):87–117. [PubMed: 16808729]
18. Cyr M, Beaulieu JM, Laakso A, Sotnikova TD, Yao WD, Bohn LM, et al. Sustained elevation of extracellular dopamine causes motor dysfunction and selective degeneration of striatal GABAergic neurons. *Proc Natl Acad Sci U S A.* 2003 Sep 16; 100(19):11035–11040. [PubMed: 12958210]
19. Xu W, Zhu JP, Angulo JA. Induction of striatal pre- and postsynaptic damage by methamphetamine requires the dopamine receptors. *Synapse.* 2005 Nov; 58(2):110–121. [PubMed: 16088948]
20. Khan ZU, Mrzljak L, Gutierrez A, de la Calle A, Goldman-Rakic PS. Prominence of the dopamine D2 short isoform in dopaminergic pathways. *Proc Natl Acad Sci U S A.* 1998 Jun 23; 95(13):7731–7736. [PubMed: 9636219]
21. Buckholtz JW, Treadway MT, Cowan RL, Woodward ND, Li R, Ansari MS, et al. Dopaminergic network differences in human impulsivity. *Science.* 2010 Jul 30; 329(5991):532. [PubMed: 20671181]
22. Wolf ME, Roth RH. Autoreceptor regulation of dopamine synthesis. *Ann N Y Acad Sci.* 1990; 604:323–343. [PubMed: 2171398]
23. Wong DF, Kuwabara H, Schretlen DJ, Bonson KR, Zhou Y, Nandi A, et al. Increased occupancy of dopamine receptors in human striatum during cue-elicited cocaine craving. *Neuropsychopharmacology.* 2006 Dec; 31(12):2716–2727. [PubMed: 16971900]
24. Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Childress AR, et al. Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. *J Neurosci.* 2006 Jun 14; 26(24):6583–6588. [PubMed: 16775146]
25. Breiter HC, Gollub RL, Weisskoff RM, Kennedy DN, Makris N, Berke JD, et al. Acute effects of cocaine on human brain activity and emotion. *Neuron.* 1997 Sep; 19(3):591–611. [PubMed: 9331351]
26. Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M, O'Brien CP. Limbic activation during cue-induced cocaine craving. *Am J Psychiatry.* 1999 Jan; 156(1):11–18. [PubMed: 9892292]
27. Garavan H, Pankiewicz J, Bloom A, Cho JK, Sperry L, Ross TJ, et al. Cue-induced cocaine craving: neuroanatomical specificity for drug users and drug stimuli. *Am J Psychiatry.* 2000 Nov; 157(11):1789–1798. [PubMed: 11058476]
28. Maas LC, Lukas SE, Kaufman MJ, Weiss RD, Daniels SL, Rogers VW, et al. Functional magnetic resonance imaging of human brain activation during cue-induced cocaine craving. *Am J Psychiatry.* 1998 Jan; 155(1):124–126. [PubMed: 9433350]
29. Volkow ND, Wang GJ, Fowler JS, Hitzemann R, Angrist B, Gatley SJ, et al. Association of methylphenidate-induced craving with changes in right striato-orbitofrontal metabolism in cocaine abusers: implications in addiction. *Am J Psychiatry.* 1999 Jan; 156(1):19–26. [PubMed: 9892293]
30. Grant S, London ED, Newlin DB, Villemagne VL, Liu X, Contoreggi C, et al. Activation of memory circuits during cue-elicited cocaine craving. *Proc Natl Acad Sci U S A.* 1996 Oct 15; 93(21):12040–12045. [PubMed: 8876259]
31. Bonson KR, Grant SJ, Contoreggi CS, Links JM, Metcalfe J, Weyl HL, et al. Neural systems and cue-induced cocaine craving. *Neuropsychopharmacology.* 2002 Mar; 26(3):376–386. [PubMed: 11850152]
32. Hommer DW. Functional imaging of craving. *Alcohol Res Health.* 1999; 23(3):187–196. [PubMed: 10890814]
33. Tabibnia G, Monterosso JR, Baicy K, Aron AR, Poldrack RA, Chakrapani S, et al. Different forms of self-control share a neurocognitive substrate. *J Neurosci.* 2011 Mar 30; 31(13):4805–4810. [PubMed: 21451018]
34. First, MB.; Spitzer, RL.; Gibbon, M.; Williams, JB. *The Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-IP).* Washington, DC: American Psychiatric Press; 1995.
35. Ashburner J, Friston KJ. Voxel-based morphometry--the methods. *Neuroimage.* 2000 Jun; 11(6 Pt 1):805–821. [PubMed: 10860804]

36. Coupe P, Yger P, Barillot C. Fast non local means denoising for 3D MR images. *Med Image Comput Comput Assist Interv.* 2006; 9(Pt 2):33–40. [PubMed: 17354753]
37. Rajapakse JC, Giedd JN, Rapoport JL. Statistical approach to segmentation of single-channel cerebral MR images. *IEEE Trans Med Imaging.* 1997 Apr; 16(2):176–186. [PubMed: 9101327]
38. Manjon JV, Tohka J, Garcia-Marti G, Carbonell-Caballero J, Lull JJ, Marti-Bonmati L, et al. Robust MRI brain tissue parameter estimation by multistage outlier rejection. *Magn Reson Med.* 2008 Apr; 59(4):866–873. [PubMed: 18383286]
39. Cuadra MB, Cammoun L, Butz T, Cuisenaire O, Thiran JP. Comparison and validation of tissue modelization and statistical classification methods in T1-weighted MR brain images. *IEEE Trans Med Imaging.* 2005 Dec; 24(12):1548–1565. [PubMed: 16350916]
40. Ashburner J. A fast diffeomorphic image registration algorithm. *Neuroimage.* 2007 Oct 15; 38(1): 95–113. [PubMed: 17761438]
41. Mukherjee J, Yang ZY, Das MK, Brown T. Fluorinated benzamide neuroleptics--III. Development of (S)-N-[(1-allyl-2-pyrrolidinyl)methyl]-5-(3-[18F]fluoropropyl)-2, 3-dimethoxybenzamide as an improved dopamine D-2 receptor tracer. *Nucl Med Biol.* 1995 Apr; 22(3):283–296. [PubMed: 7627142]
42. Ardekani BA, Braun M, Hutton BF, Kanno I, Iida H. A fully automatic multimodality image registration algorithm. *J Comput Assist Tomogr.* 1995 Jul-Aug; 19(4):615–623. [PubMed: 7622696]
43. Zald DH, Woodward ND, Cowan RL, Riccardi P, Ansari MS, Baldwin RM, et al. The interrelationship of dopamine D2-like receptor availability in striatal and extrastriatal brain regions in healthy humans: a principal component analysis of [18F]fallypride binding. *Neuroimage.* 2010 May 15; 51(1):53–62. [PubMed: 20149883]
44. Woolrich MW, Jbabdi S, Patenaude B, Chappell M, Makni S, Behrens T, et al. Bayesian analysis of neuroimaging data in FSL. *Neuroimage.* 2009 Mar; 45(1 Suppl):S173–S186. [PubMed: 19059349]
45. Lammertsma AA, Hume SP. Simplified reference tissue model for PET receptor studies. *Neuroimage.* 1996 Dec; 4(3 Pt 1):153–158. [PubMed: 9345505]
46. Mukherjee J, Christian BT, Dunigan KA, Shi B, Narayanan TK, Satter M, et al. Brain imaging of 18F-fallypride in normal volunteers: blood analysis, distribution, test-retest studies, and preliminary assessment of sensitivity to aging effects on dopamine D-2/D-3 receptors. *Synapse.* 2002 Dec 1; 46(3):170–188. [PubMed: 12325044]
47. Wu Y, Carson RE. Noise reduction in the simplified reference tissue model for neuroreceptor functional imaging. *J Cereb Blood Flow Metab.* 2002 Dec; 22(12):1440–1452. [PubMed: 12468889]
48. Bello EP, Mateo Y, Gelman DM, Noain D, Shin JH, Low MJ, et al. Cocaine supersensitivity and enhanced motivation for reward in mice lacking dopamine D2 autoreceptors. *Nat Neurosci.* 2011 Aug; 14(8):1033–1038. [PubMed: 21743470]
49. Anker JJ, Perry JL, Gliddon LA, Carroll ME. Impulsivity predicts the escalation of cocaine self-administration in rats. *Pharmacol Biochem Behav.* 2009 Sep; 93(3):343–348. [PubMed: 19490925]
50. Tarter RE, Kirisci L, Habeych M, Reynolds M, Vanyukov M. Neurobehavior disinhibition in childhood predisposes boys to substance use disorder by young adulthood: direct and mediated etiologic pathways. *Drug Alcohol Depend.* 2004 Feb 7; 73(2):121–132. [PubMed: 14725951]
51. Vidal-Infer A, Arenas MC, Daza-Losada M, Aguilar MA, Minarro J, Rodriguez-Arias M. High novelty-seeking predicts greater sensitivity to the conditioned rewarding effects of cocaine. *Pharmacol Biochem Behav.* 2012 Jul; 102(1):124–132. [PubMed: 22507913]
52. Belin D, Berson N, Balado E, Piazza PV, Deroche-Gamonet V. High-novelty-preference rats are predisposed to compulsive cocaine self-administration. *Neuropsychopharmacology.* 2011 Feb; 36(3):569–579. [PubMed: 20980989]
53. Zald DH, Cowan RL, Riccardi P, Baldwin RM, Ansari MS, Li R, et al. Midbrain dopamine receptor availability is inversely associated with novelty-seeking traits in humans. *J Neurosci.* 2008 Dec 31; 28(53):14372–14378. [PubMed: 19118170]

54. Calipari ES, Sun H, Eldeeb K, Luessen DJ, Feng X, Howlett AC, et al. Amphetamine self-administration attenuates dopamine d2 autoreceptor function. *Neuropsychopharmacology*. 2014 Jul; 39(8):1833–1842. [PubMed: 24513972]
55. Kohno M, Morales AM, Ghahremani DG, Helleman G, London ED. Risky decision making, prefrontal cortex, and mesocorticolimbic functional connectivity in methamphetamine dependence. *JAMA Psychiatry*. 2014 Jul 1; 71(7):812–820. [PubMed: 24850532]
56. Mukherjee J, Yang ZY, Brown T, Lew R, Wernick M, Ouyang X, et al. Preliminary assessment of extrastriatal dopamine D-2 receptor binding in the rodent and nonhuman primate brains using the high affinity radioligand, 18F-fallypride. *Nucl Med Biol*. 1999 Jul; 26(5):519–527. [PubMed: 10473190]
57. Boileau I, Payer D, Houle S, Behzadi A, Rusjan PM, Tong J, et al. Higher binding of the dopamine D3 receptor-preferring ligand [11C]-(+)-propyl-hexahydro-naphtho-oxazin in methamphetamine polydrug users: a positron emission tomography study. *J Neurosci*. 2012 Jan 25; 32(4):1353–1359. [PubMed: 22279219]
58. Gurevich EV, Joyce JN. Distribution of dopamine D3 receptor expressing neurons in the human forebrain: comparison with D2 receptor expressing neurons. *Neuropsychopharmacology*. 1999 Jan; 20(1):60–80. [PubMed: 9885786]
59. Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Childress AR, et al. Dopamine increases in striatum do not elicit craving in cocaine abusers unless they are coupled with cocaine cues. *Neuroimage*. 2008 Feb 1; 39(3):1266–1273. [PubMed: 18024160]
60. McFarland K, Davidge SB, Lapish CC, Kalivas PW. Limbic and motor circuitry underlying footshock-induced reinstatement of cocaine-seeking behavior. *J Neurosci*. 2004 Feb 18; 24(7):1551–1560. [PubMed: 14973230]
61. McFarland K, Lapish CC, Kalivas PW. Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. *J Neurosci*. 2003 Apr 15; 23(8):3531–3537. [PubMed: 12716962]
62. Nakama H, Chang L, Fein G, Shimotsu R, Jiang CS, Ernst T. Methamphetamine users show greater than normal age-related cortical gray matter loss. *Addiction*. 2011 Mar 25.
63. Morales AM, Ghahremani DG, Kohno M, Helleman G, London ED. Cigarette Exposure, Dependence and Craving are Related to Insula Thickness in Young Adult Smokers. *Neuropharmacology*. 2014
64. Wrase J, Makris N, Braus DF, Mann K, Smolka MN, Kennedy DN, et al. Amygdala volume associated with alcohol abuse relapse and craving. *Am J Psychiatry*. 2008 Sep; 165(9):1179–1184. [PubMed: 18593776]
65. Koob GF, Volkow ND. Neurocircuitry of addiction. *Neuropsychopharmacology*. 2010 Jan; 35(1):217–238. [PubMed: 19710631]
66. Salo R, Ursu S, Buonocore MH, Leamon MH, Carter C. Impaired prefrontal cortical function and disrupted adaptive cognitive control in methamphetamine abusers: a functional magnetic resonance imaging study. *Biol Psychiatry*. 2009 Apr 15; 65(8):706–709. [PubMed: 19136097]
67. Nestor LJ, Ghahremani DG, Monterosso J, London ED. Prefrontal hypoactivation during cognitive control in early abstinent methamphetamine-dependent subjects. *Psychiatry Res*. 2011 Dec 30; 194(3):287–295. [PubMed: 22047731]
68. Drummond DC. Theories of drug craving, ancient and modern. *Addiction*. 2001 Jan; 96(1):33–46. [PubMed: 11177518]
69. Cadet JL, Krasnova IN. Molecular bases of methamphetamine-induced neurodegeneration. *Int Rev Neurobiol*. 2009; 88:101–119. [PubMed: 19897076]

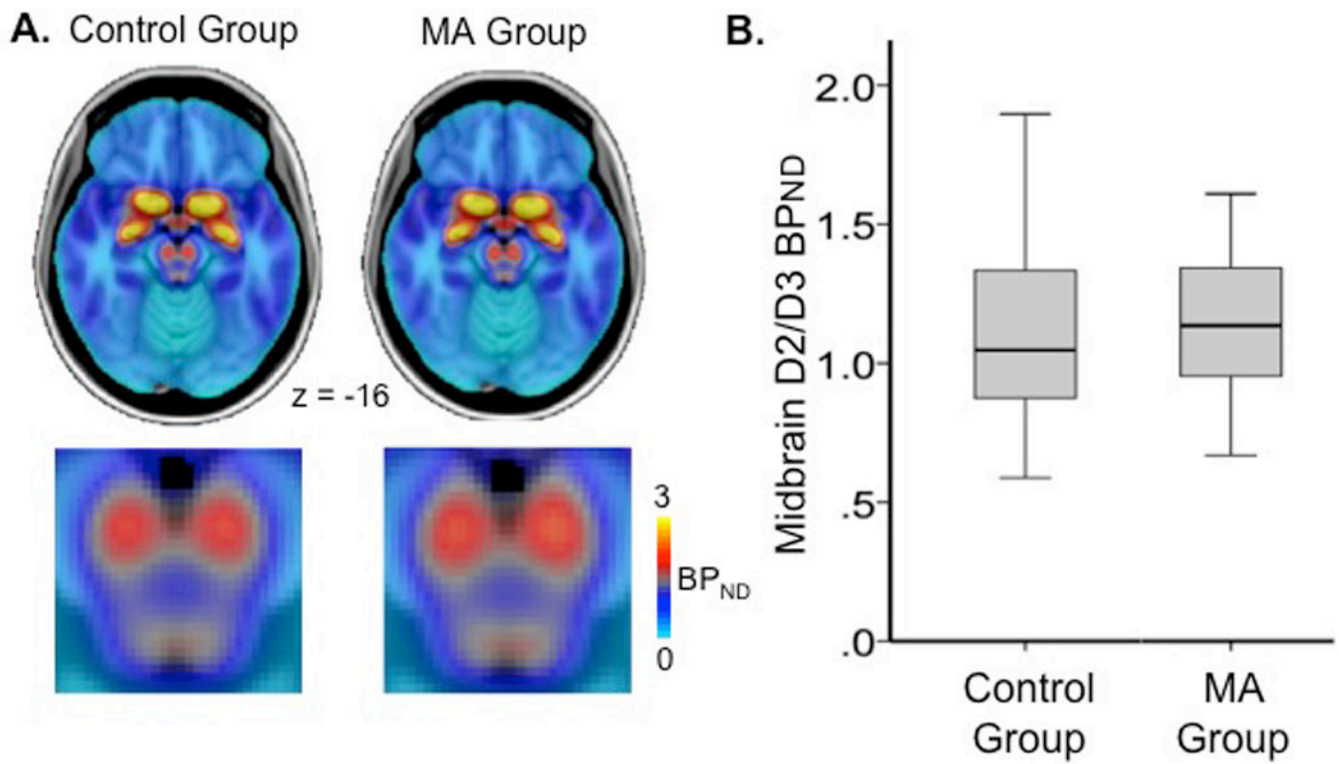


Figure 1. Midbrain D2/D3 BP_{ND} in control and methamphetamine groups

(A) In control and methamphetamine (MA) groups, maps depict average midbrain D2/D3 BP_{ND} across the whole brain with a closer view of the midbrain. The right hemisphere is displayed on the left side of the image. (B) Box plots depict average midbrain D2/D3 BP_{ND} in methamphetamine and control groups. Analysis of covariance, with age, sex, frequency of marijuana use, and smoking status as covariates, revealed no significant group differences in midbrain D2/D3 BP_{ND}.

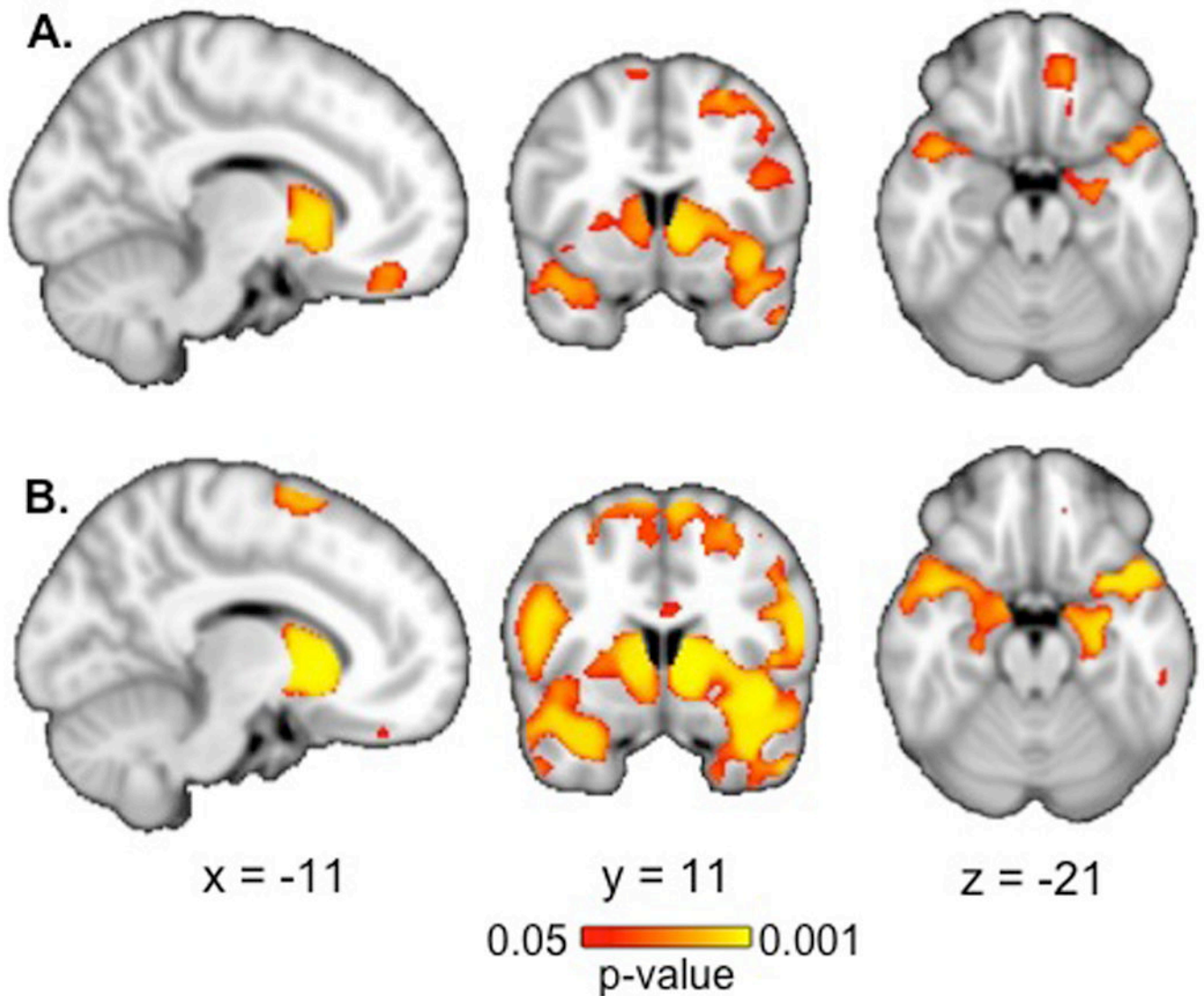
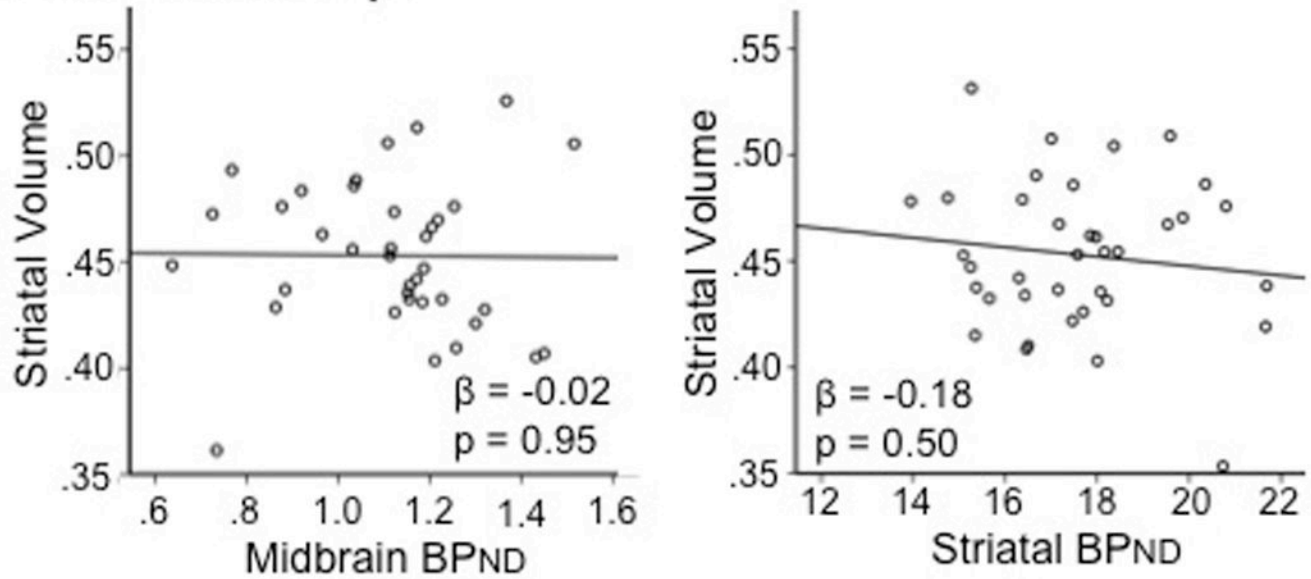


Figure 2. Associations between midbrain D2/D3 BP_{ND} and gray-matter volume

(A) Statistical maps indicate brain regions where there was a significant group by midbrain D2/D3 BP_{ND} interaction on gray-matter volume. Nonparametric analysis of covariance, including age, sex, frequency of marijuana and smoking status as covariates, showed that the relationship between gray-matter volume and midbrain D2/D3 BP_{ND} is greater methamphetamine users than controls in the striatum, insula, hippocampus and dorsolateral, orbitofrontal, inferior frontal and temporal cortices ($p < 0.05$, whole-brain correction for multiple comparisons). (B) Brain regions where midbrain D2/D3 BP_{ND} was positively associated with gray-matter volume in the methamphetamine group ($p < 0.05$, whole-brain correction for multiple comparisons). See Supplemental Table 1 for complete list of brain regions. The right hemisphere is displayed on the left side of the image.

A. Control Group



B. Methamphetamine Group

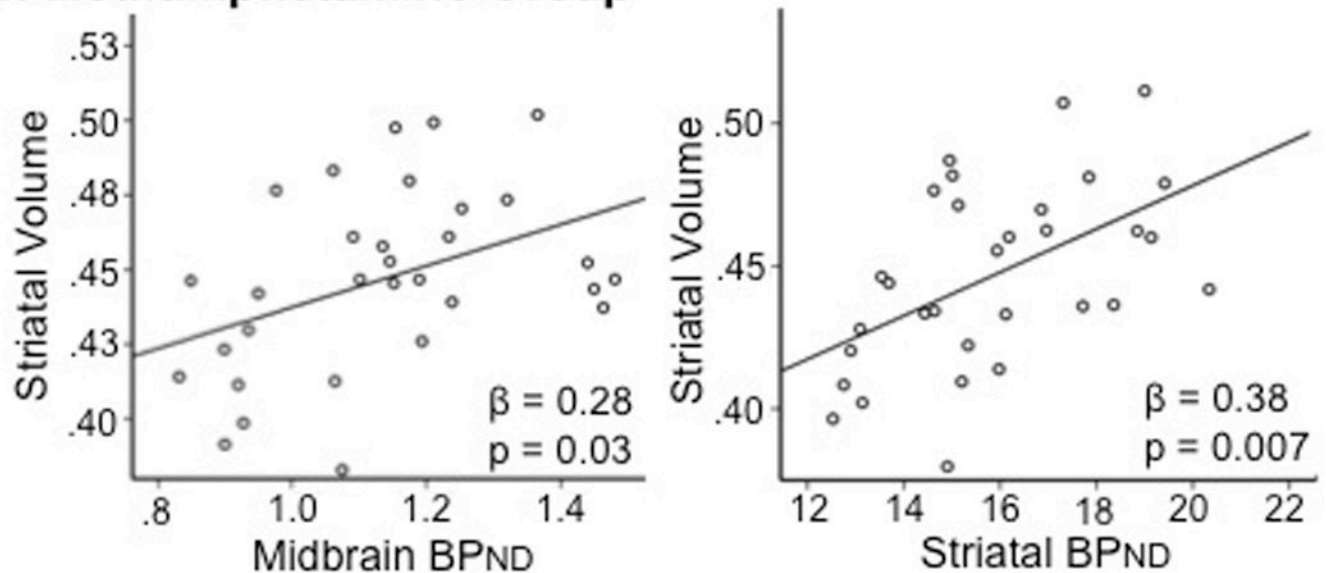


Figure 3. Associations between dopamine D2/D3 BP_{ND} and striatal volume

(A) Scatter plots show that in the control group, midbrain and striatal D2/D3 BP_{ND} were not associated with striatal volume when included in the same linear regression along with age, sex, frequency of marijuana use, and smoking status. (B) In contrast, midbrain and striatal D2/D3 BP_{ND} were both associated with striatal volume when included in the same linear regression along with age, sex, frequency of marijuana use and smoking status in the methamphetamine group. Values in the scatter plots are adjusted for all other covariates in the statistical model.

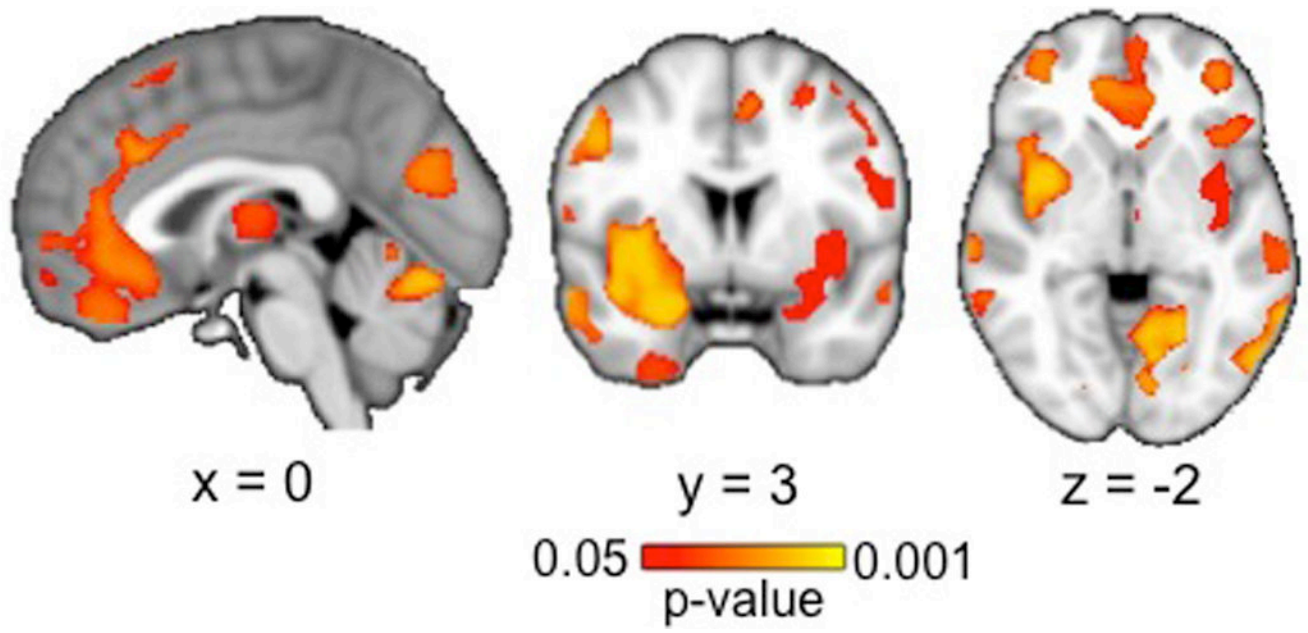


Figure 4. Craving for methamphetamine is negatively correlated with gray-matter volume
Brain regions where there was a negative association between gray-matter volume and methamphetamine craving. Statistical maps generated using permutation testing with sex, frequency of methamphetamine use and days since last methamphetamine use as covariates ($p < 0.05$, whole-brain correction for multiple comparisons). See Supplemental Table 3 for complete list of brain regions. The right hemisphere is displayed on the left side of the image.

Table 1

Participant Characteristics

Demographic and Drug Use Characteristics ^a	Methamphetamine Dependent Group (n=58)	Subset with [¹⁸ F] Fallypride BP _{ND}	
		Healthy Control Group (n=37)	Methamphetamine Dependent Group (n=31)
Age	32.8 ± 9.2	36.8 ± 1.4	33.2 ± 1.7
Sex (# Male / # Female)	28/30	24/13	15/16
Frequency of alcohol use ^b	6.1 ± 8.6	4.4 ± 1.1	4.2 ± 1.2
Average # of alcoholic drinks per week	1.8 ± 0.3	1.7 ± 0.4	1.5 ± 0.3
Frequency of marijuana use ^{a*}	4.2 ± 8.4	0.3 ± 0.3	4.0 ± 1.4
Number of cigarette smokers ^{**}	54	21	26
Pack year smoking history	9.9 ± 12.9	6.6 ± 1.7	10.6 ± 2.5
Frequency of methamphetamine use ^a	22.1 ± 7.8	n/a	20.1 ± 1.4
Years of heavy methamphetamine use ^c	7.9 ± 6.8	n/a	9.4 ± 1.4

^a Data presented are mean ± standard error unless otherwise indicated

^b Number of days of drug use in the 30 days preceeding study entry

^c Data missing for 3 subjects; heavy use defined as 3 times per week or at least one 2-day binges per week.

* Difference between methamphetamine and control groups with [¹⁸F]fallypride BP_{ND} (p < 0.05 by Student's t-test)

** Difference between methamphetamine and control groups with [¹⁸F]fallypride BP_{ND} (p < 0.05 by Chi-squared test)